





## Article

# Lecciana, a New Low-Vigour Olive Cultivar Suitable for Super High Density Orchards and for Nutraceutical EVOO Production

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**Abstract:** Cultivar is the key factor for sustainability of the olive super high density planting system (SHD). ‘Lecciana’ is a new olive cultivar for oil production obtained in 1998 by a controlled cross between cv. Arbosana (♀) and cv. Leccino (♂) in a breeding program as part of an international research agreement between Agromillora Iberia S.L.U. and University of Bari. ‘Lecciana’ is the first olive cultivar of Italian descent suitable for SHD, featuring all the vegetative and productive traits required for efficient, sustainable olive growing intensification. Thanks to low vigor, early bearing (3rd year after planting), high yield efficiency (about 0.5 kg of fruits cm<sup>-2</sup> of trunk section area) and good fruit size (3.5 g), ‘Lecciana’ could be planted with tree densities over 1,200 trees per hectare for an efficient continuous mechanical harvesting. High frost resistance, very low pistil abortion (3%), high fruit set (3%), oil content (over 19% fw) and, above all, good unsaturated fatty acids profile, polyphenols content (over 450 mg kg<sup>-1</sup>) and fruitiness median are the main distinctive characters of this new cultivar. The oils of ‘Lecciana’ fall into the category ‘nutraceutical EVOOs’ which can benefit from the specific functional health claim.

**Keywords:** breeding program; molecular markers; morphological markers; genetic sterility; yield efficiency; fatty acid composition; polyphenols; sustainable intensification; health claim



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## 1. Introduction

Traditional olive orchards are increasingly devoted to landscape safeguarding and cultural heritage enhancement [1,2], but they have lost in general their economic sustainability [3–5]. In order to face the increasing production costs and the scarcity of manual labor, 25 years ago olive growing moved to super high density planting systems (SHD), characterized by hedgerow tree canopies suitable for continuous mechanical harvesting by straddle machines. Currently, more than 400,000 ha of SHD olive orchards are scattered all over the world, half in Spain where these new systems were born [6]. Intensification of olive planting systems represents a very interesting approach to enhance olive orchard environmental sustainability, reducing water footprint and other agronomical inputs [7–9]. Making the new olive industry economically and environmentally sustainable is the main challenge for the future, and it can only be achieved through significant increases of orchard efficiency [10,11]. The agronomic and environmental sustainability of these new planting systems depend strongly on the availability of cultivars with specific traits: first of all, low vigor, early bearing and high yield efficiency [3]. Cultivars showing all these traits are currently a limited varietal pool. At first, Arbequina, Arbosana and Koroneiki were the only three varieties cultivated in SHD orchards, on which SHD systems have been calibrated [12,13]. These cultivars belong to the traditional Spanish and Greek olive platform [14]. Later, Spanish breeding programs added ‘Sikitita’ (patented in 2007) and

'Oliana' (patented in 2012), two novel cultivars obtained by controlled crosses of Spanish cultivars: 'Piqual' × 'Arbequina' and 'Arbequina' × 'Arbosana', respectively [15,16]. In the last decades the Italian breeding programs on olive tree licensed two new cultivars suggested for SHD orchards: FS-17/Favolosa (patented in 1993) and Urano/Tosca (patented in 1998 and 2012), both obtained by free pollination of cv. Frantoio [17,18]. The former shows yield parameters fitting an olive genotype suitable for SHD orchards, such as early and high fruit bearing traits, but it has unwanted vegetative traits, first of all a spreading habit and sparse canopy density [19,20]. On the contrary, the latter provides interesting vegetative characters, such as weak vigor and dense canopy, but features erratic and inadequate yields [21,22]. The diffusion of SHD olive orchards in Italy is still small, being limited to some 4,000 ha (i.e., 0.3% of the national growing area) [6]. The main constraint seems to be the limited availability of suitable cultivars, currently all of foreign origin. Actually, an olive genotype of Italian origin providing all the vegetative and productive traits fitting for SHD growth is still lacking. At the same time, the international olive oil market is characterized by increasing competition, which encourages producers to differentiate their products [23]. Evidence from the United States and Europe indicates that the introduction of health claims can increase the market share for different nutraceutical products [24]. Among the list of claims approved by the EFSA, four are applicable to EVOO (European Commission, 2014; European Community, 432/2012). Three of the four claims are authorized as functional health claims (Art.13 (1) of Regulation (EC) No. 1924/2006). In particular, one of them is specific to olive oil and relates to the level of 'polyphenol' compounds. The claimed health function concerns the protection of blood lipids from oxidative stress. The claim "olive oil polyphenols" can be used only for an olive oil that contains at least 300–350 mg·kg<sup>-1</sup> of phenolic compounds [25]. The total concentration of phenolic compounds in oils belonging to the class marketable as EVOO varies widely, between 40 mg·kg<sup>-1</sup> and 1,000 mg·kg<sup>-1</sup> [26]. The cultivated genotype also has a great effect on nutraceutical value of EVOOs extracted in SHD olive orchards [27,28]. In all cases, the polyphenols of EVOO make it more stable [29] and healthy and contribute to its sensory characteristics [30]. Providing a growing global population with healthy diets from sustainable agrifood systems is an immediate challenge [31]. At the end of the last century, Agromillora Iberia S.L.U. started a controlled crossbreeding program aimed to obtain new cultivated olive genotypes suited for SHD planting systems, while coping with high agronomic efficiency and with market demands of nutraceutical oils as well.

## 2. Origin and Selection

In 1998 a controlled cross between cv. Arbosana (♀) and cv. Leccino (♂) was made in Catalonia (Spain) in the context of the Agromillora Iberia S.L.U. crossbreeding program using Italian parents, as part of an international research agreement with the University of Bari (Italy). Before the discovery of its full suitability for SHD orchards, cv. Arbosana was a critically endangered olive genotype: indeed in 1995 only 200 trees remained in the Penedés region of Spain where it is autochthonous [32]. The large use of 'Arbosana' as a low vigor valuable parent in most of the international breeding programs is a successful model to safeguard the biodiversity of olive cultivars at risk of extinction [3]. On the contrary, 'Leccino' is a well-known Italian cultivar spread worldwide, which oils are particularly appreciated for its organoleptic characteristics and high polyphenol contents [33]. The first seedlings screening in the nursery was based on two parameters: resistance to olive leaf spot [*Spilocaea oleagina* (Cast.) Hugh] and early bearing. The former was considered the key disease, even if the following agronomic management proved useful to control it successfully. The latter is the key agronomic aspect to keep for suitability in SHD planting systems [34]. Then, tree vigor and oil content were the parameters used for the second screening in nursery, applied to those seedlings that passed the first one. Subsequently, the selected progenies were multiplied by semi-hardwood cutting and planted in test orchards located in different growing areas, using the Arbequina, Arbosana and Koroneiki cultivars as reference genotypes. This first field evaluation occurred for six bearing years; olive

leaf spot, olive fly (*Bactrocera oleae*, Rossi), olive tree mangle (*Pseudomonas savastanoi* pv. *savastanoi*), frost resistance and oil quality were the parameters applied for the selection. Finally, only the promising progenies were multiplied and planted in olive farms, as pre-commercial selections. OAC9806-10, coded as 'Lecciana', was identified in the commercial farms that tested it starting from 2013 in Italy. 'Lecciana' is not a GMO.

### 3. Description

#### 3.1. Materials and Methods

##### 3.1.1. Site and Orchard

The comparison orchard is located in Foggia (Southern Italy, 41°40'70" N, 15°67'70" E, 39 m a.s.l.). The climate is typically Mediterranean, with an annual rainfall of 522 mm concentrated from autumn to spring; the lowest amount of monthly rainfall occurs in August (21 mm) and the highest one (60 mm) in December. The annual average temperature is 16.6 °C; the hottest month is July (27.3 °C) and the coldest one is January (7.2 °C). The soil is clay-loam, with pH 6.8 and properly expanded roots. One thousand trees of the pre-commercial selection OAC9806-10 and the same number of trees of cv. Arbequina, multiplied by semi-hardwood cutting, were planted in May 2013, with a tree spacing of 4.0 × 1.5 m (1670 trees ha<sup>-1</sup>) and a N-S row orientation. Yield, vigor and yield efficiencies were compared to 'Arbequina' as the standard variety currently used for SHD olive orchards. A randomized block design with three replicates was used; each block consisted of five rows per cultivar (ten rows per block), in which the three central rows were the sampling area. The trees were trained according to Smarttree<sup>®</sup> criteria (SHD 2.0) and they were managed using common practices in the area; drip lines were used and controlled deficit irrigation was applied, with a mean seasonal irrigation volume of 3000 m<sup>3</sup>·ha<sup>-1</sup>. The plots were supplied annually with 110 kg·ha<sup>-1</sup> of N, 50 kg·ha<sup>-1</sup> of P and 120 kg·ha<sup>-1</sup> of K.

##### 3.1.2. Molecular Markers

Fresh leaves were collected from both parents (Arbosana, Leccino) and F<sub>1</sub> (Lecciana). DNA extraction was performed following the protocol of [35]. DNA quality and concentration were assessed through 1% agarose gel electrophoresis and a Nano-Drop<sup>™</sup>2000C spectrophotometer (Thermo Scientific, Waltham, MA, USA). Samples were genotyped using two highly polymorphic simple sequence repeats (SSR): EMO 90 [36] and GAPU101 [37], selected among a panel of 12 SSR markers. PCR reactions were performed in a final volume of 12 µL according to [38]. In order to verify PCR efficiency, PCR products were checked by 1% agarose gel electrophoresis. The amplification products were detected by an ABI PRISM 3100 Avant Genetic Analyzer automatic sequencer (Applied Biosystems, Foster City, CA, USA), and the allele sizes were assigned through the GeneMapper Software version 3.7 (Life Technologies, Carlsbad, CA, USA). GeneScan 600 LIZ (Applied Biosystems) was used as internal molecular size standard.

##### 3.1.3. Morphological Markers

In order to assess the distinctness of this new cultivar, we selected 26 morphological markers with high genetic expression according to UPOV Guidelines [39], that require a minimum quantity of five trees (§ 2.3) to be tested for a minimum of two growing cycles (§ 3.1), normally at one place (§ 3.2). The marker/characteristic number referring to UPOV Table TG/99/4 is reported. All markers were determined on 10 healthy bearing trees of 'Lecciana', randomly chosen in each experimental plot among the trees of the sampling area. The test was performed for four growing cycles (2015–2018).

##### 3.1.4. Genetic Sterilities and Blooming Phenogram

Pistil abortion and self-incompatibility are undoubtedly the main varietal characteristics responsible for the fruit setting of olive tree [40]. These two genetic sterilities were evaluated on the same trees of 'Lecciana' used for the morphological markers for two grow-

ing cycles (2015–2016). The percentage of gynosterility was evaluated on 60 inflorescences per tree taken from three different canopy layers (0.5–1.0 m; 1.0–1.5 m; 1.5–2.0 m) at the start of blooming. Self-incompatibility was assessed by the methodology already described by [41] on 600 inflorescences per tree, divided into two groups for each side of canopy (East and West): the inflorescences of the first group were isolated with non-woven bags prior to anthesis and were left to unassisted self-pollination by wind buffeting of branches throughout the blooming time. The inflorescences of the second group were left to open-pollination by wind. The fruit set was evaluated at veraison. The open flowers percentage, left to open-pollination, was measured weekly in order to describe the blooming phenogram (10% = start, 50% = full, 90% = end).

### 3.1.5. Ripening Indexes and Harvesting Time

In order to establish the optimal time to harvest ‘Lecciana’, different ripening indexes were monitored during four ripening times (expressed as days after full bloom) of two subsequent seasons (2015–2016). Fifty olives of ‘Lecciana’ from each tree were randomly sampled to determine eight fruit ripening indices—fresh and dry weight (g), detachment force (cN), firmness ( $\text{cN cm}^{-2}$ ), Jaen pigmentation index (n), detachment index ( $\text{N g}^{-1}$ ), oil content (% fw) and humidity (%)—following the methodology reported by [42]. In hedgerow olive trees the efficiency of continuous mechanical harvesting changes in time especially as a function of fruits detachment force and fresh weight: these are, in fact, the parameters most correlated to the harvesting yield [43]. The detachment index, defined as the ratio between these two indexes, represents the main index that characterizes the SHD olive orchard mechanical harvesting efficiency [44].

### 3.1.6. Yield, Vigor and Yield Efficiencies

These agronomic parameters were evaluated on the same trees of ‘Lecciana’ used for the morphological markers and on the same number of trees of ‘Arbequina’, for four growing cycles (2015–2018). The fruits were harvested when the Jaen index reached values between 2 and 3. At harvesting time all the fruits for each tree were collected to measure the fruit yield (FY;  $\text{kg tree}^{-1}$ ). Canopy volume ( $\text{m}^3 \text{ tree}^{-1}$ ) was calculated considering the parallelepiped volume as:

$$(\text{tree high} - 0.5 \text{ m}) \times \text{tree depth} \times 1.5$$

where 0.5 is distance from the soil where branches start and 1.5 is the width between trees on the row. Trunk diameter (TD; cm) was measured after harvesting at 0.5 m from the soil. Tree section area (TS;  $\text{cm}^2$ ) was calculated as:

$$\text{TS} = \pi \text{TD}^2 / 4.$$

Fruit yield efficiency (FYE;  $\text{kg cm}^{-2}$ ) was calculated as:

$$\text{FYE} = \text{FY} / \text{TS}.$$

Oil content (%; OC fw) and olive moisture (%; OM) were calculated on the fruits harvested per tree by means of Soxhlet method. Oil yield was calculated on a fresh weight basis (OY fw;  $\text{kg fw tree}^{-1}$ ). Oil content on dry weight (OC dw;  $\text{kg dw tree}^{-1}$ ) was calculated as:

$$\text{OC dw} = \text{OC} 100 / (100 - \text{OM})$$

Oil yield efficiency on dry weight basis (OYE;  $\text{kg dw cm}^{-2}$ ) was calculated as:

$$\text{OYE} = \text{FYE OC dw}.$$

### 3.1.7. Oil Quality

The main fatty acids composition (%) of the oil samples extracted from the fruits harvested from each tree of ‘Lecciana’ and ‘Arbequina’ was determined for four growing cycles (2015–2018) following the international standard method (Regulation EC No 2568/1991 and subsequent EU amendments and additions). Total polyphenols content and median fruitiness of ‘Lecciana’ oils extracted at four ripening times in 2015 and 2016 were determined. Polyphenol content (mg caffeic acid kg<sup>-1</sup> oil) was quantified colourimetrically following the Folin-Ciocalteu method and measured spectrophotometrically at a wavelength of 726 nm. Median fruitiness was obtained by an official panel test as provided for in Regulation (EC) No 2568/1991 and (EU) subsequent amendments and additions.

### 3.2. Data Analysis

Field and lab data collected were analyzed by one and two-way analysis of variance (ANOVA) followed by a post hoc testing (Siegel-Tukey protected test) using the R 2.15.0 software (R Foundation for Statistical Computing, Boston, MA, USA); the standard error (SE) was also calculated.

## 4. Results and Discussion

### 4.1. Molecular Markers

The microsatellite analysis (SSRs) aimed to select molecular markers polymorphic between parents and to confirm the hybrid nature of the Lecciana genotype. Among the well-established SSR set used for genetic fingerprint and parentage analysis [45,46] we selected two molecular markers with simple profile and easy to follow. The profile of EMO90 and GAPU101 markers is reported in Table 1. Among them, GAPU 101 showed the most informative profile with four totally different alleles between the two parents. Lecciana showed the allele 181, coming from Arbosana and the allele 197 from the male parent Leccino.

**Table 1.** Allelic profile of two polymorphic molecular markers for ‘Lecciana’ and for its parental cultivars Arbosana and Leccino. Allele size is reported in base pairs (bps).

Genotype	EMO90		GAPU101	
Arbosana (♀)	181	183	181	187
Leccino (♂)	183	189	195	197
Lecciana (F <sub>1</sub> )	183	183	181	197

### 4.2. Morphological Markers

Morphological markers of ‘Lecciana’ are reported in Table 2. Young and adult trees (Figure 1), leaves and inflorescences on bearing shoot (Figure 2), fruit and stone (Figure 3) photos are also reported. ‘Lecciana’ is a weak vigor cultivar. Moreover, its upright growth habit and medium canopy density make it suitable for SHD orchards [47,48]. Indeed, these ‘Lecciana’ genetic characteristics lead to the optimal training form in SHD hedgerows, thus reducing intra-row competition and pruning operations [49]. The juvenile period of ‘Lecciana’ is 2 years, with a significant yield at the 3rd year after planting. A tendency to alternate bearing it was observed in some growing areas, but it is always related to a sub-optimal canopy management, in terms of pruning, water and nutrients. The standard fruit weight is 3.5 g. Considering this good fruit size and that the flesh is freestone, Lecciana is a potential table olive cultivar, even if other parameters should be considered.

**Table 2.** Main morphological markers of ‘Lecciana’. Values of four subsequent seasons. The characteristic numbers referring to UPOV table TG/99/4 are reported for each marker in brackets.

Tree	Vigor (1)	Weak
	Growth habit (2)	Upright
	Canopy density (3)	Medium
Leaf	Shape (7)	Elongated
	Blade longitudinal curvature (9)	Straight
Inflorescence	Length (11)	Short
Fruit	Length (14)	Medium
	Width (15)	Narrow
Stone	Weight (16)	Medium
	Shape (17)	Elliptic
	Size of lenticels (20)	Medium
	Number of lenticels (21)	Few
	Over color at full maturity (22)	Dark violet
	Symmetry in position A (23)	Weakly asymmetric
	Shape of apex in position A (24)	Rounded
	Nipple (25)	Absent
	Shape of base (26)	Truncate
	Shape in position B (28)	Elliptic
	Length (29)	Medium
	Width in position B (30)	Narrow
	Weight (32)	Medium
	Symmetry in position A (33)	Weakly asymmetric
	Number of grooves on basal end (35)	Between 7 and 10
	Shape of apex in position A (37)	Acute
Mucron (38)	Present	
Rugosity of surface (40)	Strong	



**Figure 1.** Young (left) and adult (right) ‘Lecciana’ trees.



**Figure 2.** Leaves and inflorescences on bearing shoot of 'Lecciana'.



**Figure 3.** Fruit and stone of 'Lecciana'.

#### 4.3. Genetic Sterilities and Blooming Phenogram

Due to different winter-spring weather patterns in 2015 and 2016, the blooming occurred in different dates that ranged from the end of April to the second decade of May. The blooming dates just shifted by 2–4 days so that the phenogram duration was 14–17 days (Table 3). Full bloom date (FB; 50% of open flowers) could be around on May 10, with a mean blooming duration of 15 days.

**Table 3.** Blooming phenogram of ‘Lecciana’ in two subsequent seasons. Full bloom corresponds to the date with 50% of open flowers.

Open Flowers (%)	2015	2016
10	May 3	April 29
50	May 12	May 9
90	May 16	May 15

The mean number of flowers per inflorescence is 14.5, without differences among positions in the canopy (Table 4). The gynosterility percentage is 3%, with the maximum values (5%) in the middle part of the canopy (Table 4). In all cases, ‘Lecciana’ shows a very low pistil abortion [50].

**Table 4.** Flower per inflorescence (n) and gynosterility (%) of cv. Lecciana as effect of inflorescence position along the canopy. Mean values of two subsequent seasons ( $\pm$ SE). Lower case letters denote statistical differences at  $p = 0.05$  (Siegel-Tukey test); ns = not significant.

Position (m)	Flowers Per Inflorescence (n)	Gynosterility (%)
1.5–2.0	15.8 $\pm$ 2.3 ns	1.68 $\pm$ 0.8 c
1.0–1.5	14.4 $\pm$ 2.0 ns	5.01 $\pm$ 2.1 a
0.5–1.0	13.2 $\pm$ 2.1 ns	2.56 $\pm$ 1.9 b
mean	14.5 $\pm$ 2.5	3.08 $\pm$ 2.2

The fruit set mean value of 0.1% by self-pollination (Table 5) points to the fact that ‘Lecciana’ is a self-incompatible cultivar, as are most olive cultivars [51]. The pollinators should be the same of the parent cultivars, i.e., Leccino and Arbosana [52]. However, in the case of very large monovarietal areas with self-incompatible cultivars, like southern Italy, Spain, Portugal, North Africa, the introduction of pollinizer cultivars to make more effective cross-pollination is of limited importance, since the availability of large amount of pollen transferred by wind from neighboring districts with different cultivars may assure naturally optimal fruit sets [53]. The association of compatible cultivars for cross-pollination retains all its importance in the case of new, small and/or isolated olive districts, such as sub-desert regions of California, Israel, Australia [41]. Open-pollination of ‘Lecciana’ gives an optimal fruit set of more than 3% on both exposures of the canopy (Table 5).

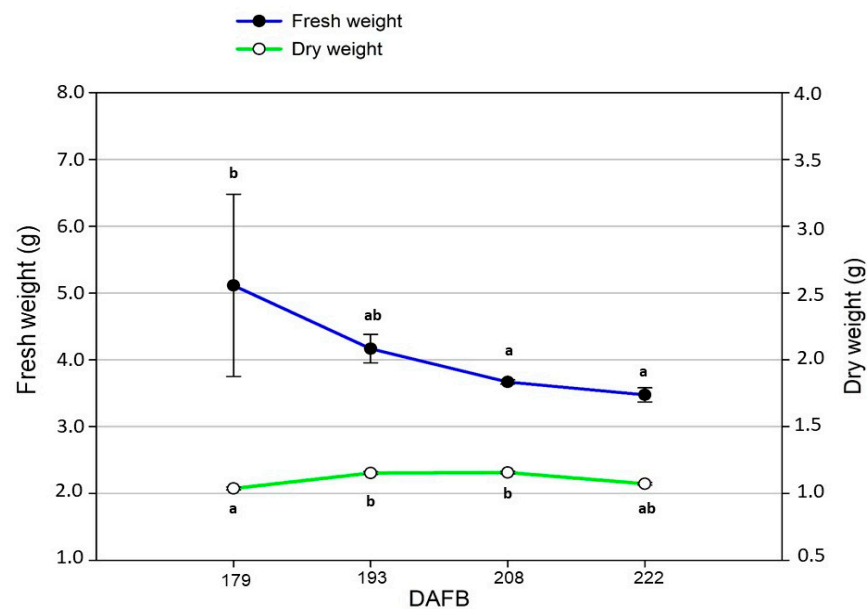
**Table 5.** Fruit set (%) of cv. Lecciana at veraison as effect of pollination type (Self/Open) and exposure (Est/West). Mean values of two subsequent seasons ( $\pm$ SE). Lower case letters denote statistical differences at  $p = 0.05$  (Siegel-Tukey test).

Pollination Type	Exposure	Fruit Set (%)	Mean
Self	Est	0.10 $\pm$ 0.05 b	0.10 $\pm$ 0.05 b
	West	0.09 $\pm$ 0.04 b	
Open	Est	2.92 $\pm$ 0.67 a	3.24 $\pm$ 0.78 a
	West	3.56 $\pm$ 0.83 a	



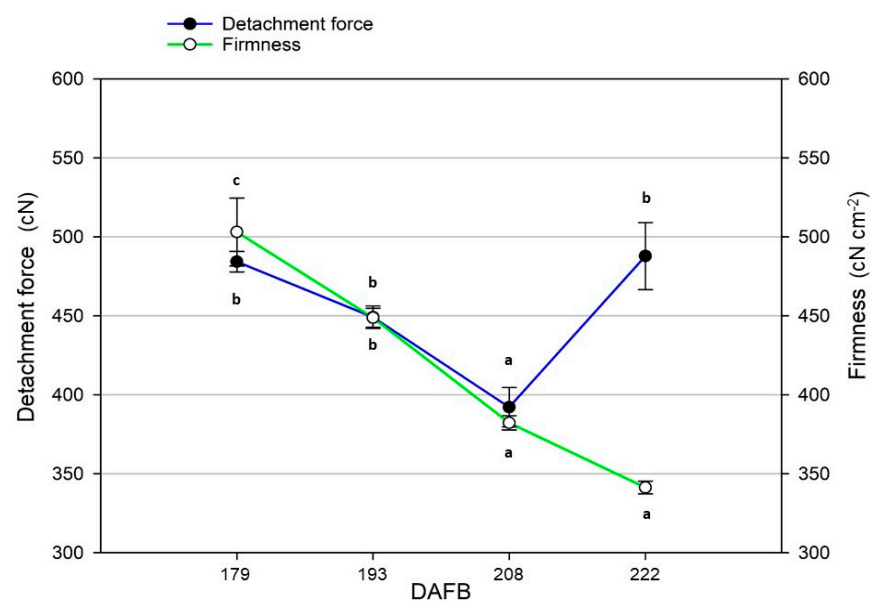
#### 4.4. Ripening Indexes and Harvesting Time

During ripening period, olives fresh and dry weight varied little (Figure 4). Fresh fruit tended to slightly reduce from 179 DAFB to 193 DAFB, when it assumed the final value of about 3.5 g. Dry weight stayed around 1.0–1.1 g during all periods.



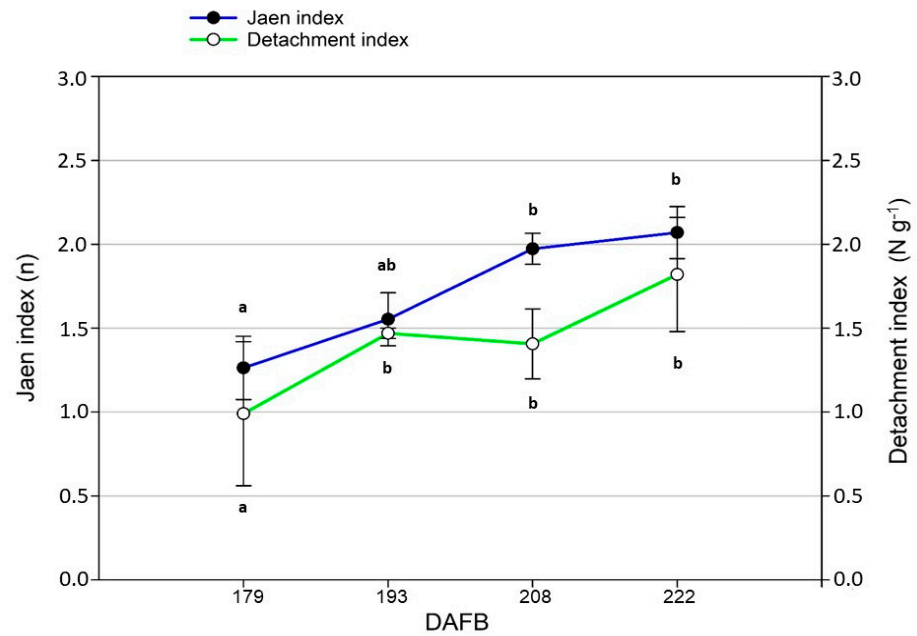
**Figure 4.** Fresh and dry weight (g) of olives of ‘Lecciana’ during ripening (DAFB = days after full bloom). Mean values of two subsequent seasons ( $\pm$ SE). Lower case letters denote statistical differences at  $p = 0.05$  (Siegel-Tukey test).

In general, detachment force and firmness decreased significantly (Figure 5). ‘Lecciana’ showed progressively reduced fruit detachment force from 484 cN to 392 cN and fruit firmness from 503 cN cm<sup>-2</sup> to 341 cN cm<sup>-2</sup>, changing from 179 DAFB to 208 DAFB, respectively. However, detachment force after it reached the minimum value at 208 DAFB tended to increase to 487 cN at 222 DAF.



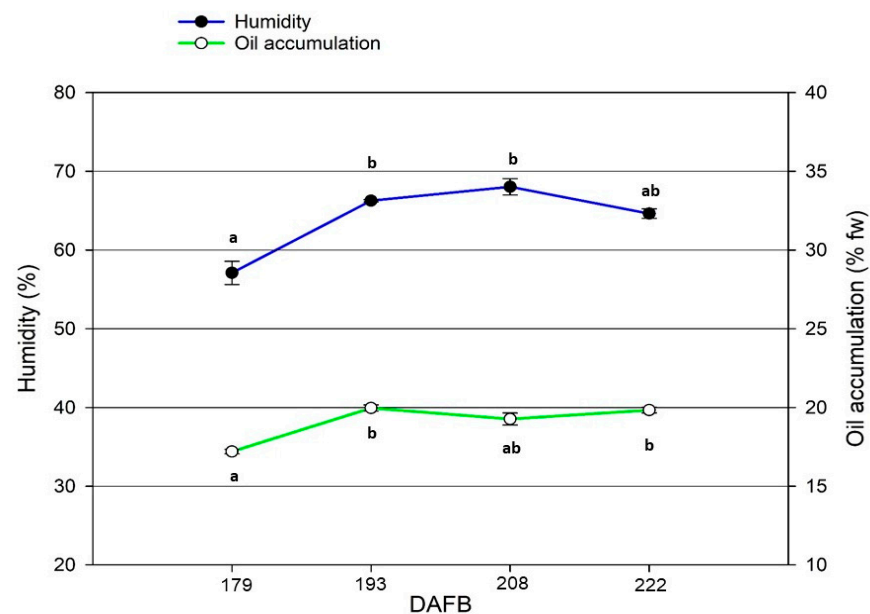
**Figure 5.** Detachment force (cN) and firmness (cN cm<sup>-2</sup>) of olives of ‘Lecciana’ during ripening (DAFB = days after full bloom). Mean values of two subsequent seasons ( $\pm$ SE). Lower case letters denote statistical differences at  $p = 0.05$  (Siegel-Tukey test).

Detachment index varied around values of  $1.5 \text{ N g}^{-1}$  to  $2.0 \text{ N g}^{-1}$  from 193 to 222 DAFB, while Jaen index increased significantly from 1.3 at 179 DAFB to 2.0 in the last sampling time (Figure 6). The mechanical harvesting efficiency is maximum (90–95%) when the detachment index is less of  $2.0 \text{ N g}^{-1}$  [42].



**Figure 6.** Jaen index ( $n$ ) and detachment index ( $\text{N g}^{-1}$ ) of olives of ‘Lecciana’ during ripening (DAFB = days after full bloom). Mean values of two subsequent seasons ( $\pm\text{SE}$ ). Lower case letters denote statistical differences at  $p = 0.05$  (Siegel-Tukey test).

Olive humidity ranged around 57% to 64%, while oil accumulation increased significantly from 17.2 % at 179 DAFB to 19.7% as mean in the last dates of ripening period. (Figure 7).



**Figure 7.** Oil accumulation (% fw) and humidity (%) in olives of ‘Lecciana’ during ripening (DAFB = days after full bloom). Mean values of two subsequent seasons ( $\pm\text{SE}$ ). Lower case letters denote statistical differences at  $p = 0.05$  (Siegel-Tukey test).

At 208 DAFB fruit drop started. Indeed at 222 DAFB an increase of detachment index (Figure 5) due to the concurrent raise of detachment force (Figure 5) and decrease of fresh weight (Figure 4) of the standing sampled olives was highlighted. However, in this last ripening period other ripening indexes, as Jaen index (Figure 6), oil content and humidity (Figure 7) did not vary, remaining significantly unchanged. These ripening pattern data are useful for assessing the optimal harvesting time for this new cultivar.

In order to maximize both the efficiency of the continuous mechanical harvesting and the olives/oil harvestable, the evaluation of the optimal harvesting time was carried out determining for 'Lecciana' the moment when: (1) the detachment index falls under the threshold value of  $2 \text{ N}\cdot\text{g}^{-1}$ , (2) the oil accumulation ends and (3) just before the fruit drop starts, so the optimal ripening time to harvest olives results between 193 and 208 DAFB, when Jaen index is 2 and detachment index is  $1.5 \text{ N}\cdot\text{g}^{-1}$  (Figure 8).



**Figure 8.** Olives of 'Lecciana' at the optimal ripening phase to harvesting, around 200 days after full bloom.

Under the prevalent environmental conditions of Apulia, the optimal harvesting time occurs during the second decade in November, just after cv. Arbequina and before cv. Arbosana [42]. At this time (around 200 DAFB) 'Lecciana' achieves the standard performances of 3.5 g of fruit fresh weight and 19.3% of oil content (fw). Moreover, the good fruit size allows efficient mechanical harvesting by both straddle harvesters and trunk shakers as well [44]. Finally, with this cultivar it is possible to cover the gap in harvesting time between cultivars Arbequina and Arbosana, thus optimizing harvester machine use.

#### 4.5. Yield, Vigor and Yield Efficiencies

'Lecciana' is more productive than 'Arbequina' in terms of both mean (about 1.0 kg olives tree<sup>-1</sup> more) and cumulated fruit yield, but it shows a +15% higher vigor than the reference cultivar in terms of canopy volume, even if much less (3%) in terms of trunk section area data (Table 6), confirming what reported in Table 2. Moreover, this new cultivar yields about 1.0% of oil content less than 'Arbequina'. Nonetheless, 'Lecciana' provides higher fruit yield efficiency (0.48 vs. 0.42 kg olives cm<sup>-2</sup>) and cumulated oil yield (4.5 vs. 4.0 kg oil fw tree<sup>-1</sup>) than the reference cultivar. Finally, 'Lecciana' and 'Arbequina' feature the same values of oil yield efficiency (0.20 kg oil dw·cm<sup>-2</sup>).

**Table 6.** Canopy volume, fruit and oil yield, trunk section area and yield efficiencies of ‘Lecciana’ compared with ‘Arbequina’ (fw = fresh weight; dw dry weight). Mean values of 4 years ( $\pm$ SE). c = cumulated values; \* = statistical significance at  $p = 0.05$  (Siegel-Tukey test); ns = not significant.

Parameters	Lecciana	Arbequina	
Canopy volume ( $\text{m}^3 \cdot \text{tree}^{-1}$ )	$6.6 \pm 1.0$	$5.7 \pm 0.6$	*
Fruit yield ( $\text{kg} \cdot \text{tree}^{-1}$ )	$6.1 \pm 1.1$	$5.0 \pm 1.2$	*
c Fruit yield ( $\text{kg} \cdot \text{tree}^{-1}$ )	$23.3 \pm 3.4$	$19.7 \pm 2.1$	*
Trunk section area ( $\text{cm}^2$ )	$48.5 \pm 0.5$	$47.0 \pm 0.7$	*
Fruit yield efficiency ( $\text{kg} \cdot \text{cm}^{-2}$ )	$0.48 \pm 0.05$	$0.42 \pm 0.08$	*
Oil content (% fw)	$19.3 \pm 1.2$	$20.5 \pm 1.5$	*
Olive Moisture (%)	$58.4 \pm 2.4$	$57.2 \pm 2.5$	ns
c Oil yield ( $\text{kg fw} \cdot \text{tree}^{-1}$ )	$4.50 \pm 0.59$	$4.04 \pm 0.36$	*
Oil yield efficiency ( $\text{kg dw} \cdot \text{cm}^{-2}$ )	$0.20 \pm 0.03$	$0.20 \pm 0.04$	ns

#### 4.6. Oil Quality

‘Lecciana’ oils showed medium contents of palmitic, palmitoleic and linoleic acid and high contents of oleic acid (Table 7). Moreover, the oils’ fatty acids profile of this new cultivar is very closer to the reference values of the Italian monovarietal extra virgin olive oils (EVOOs) [54] than that of ‘Arbequina’ oils, except for linoleic and linolenic acids.

**Table 7.** Main fatty acids composition of ‘Lecciana’ oils compared to ‘Arbequina’ ones. Mean values of 4 years ( $\pm$ SE). \* = statistical significance at  $p = 0.05$  (Siegel-Tukey test); ns = not significant. Italian monovarietal EVOOs reference values are reported.

Fatty Acids	Lecciana	Arbequina		Italian
Palmitic acid (C16:0; %)	$15.1 \pm 0.3$	$16.9 \pm 1.0$	ns	12.01
Palmitoleic acid (C16:1; %)	$1.1 \pm 0.1$	$2.0 \pm 0.3$	*	1.1
Stearic acid (C18:0; %)	$2.3 \pm 0.4$	$1.6 \pm 0.4$	*	2.2
Oleic acid (C18:1; %)	$73.7 \pm 2.4$	$70.2 \pm 1.0$	ns	73.0
Linoleic acid (C18:2; %)	$6.2 \pm 0.1$	$13.2 \pm 1.5$	*	10.3
Linolenic acid (C18:3; %)	$0.6 \pm 0.0$	$0.6 \pm 0.2$	ns	0.3

At harvesting time, around 200 DAFB, the total polyphenols content is  $460 \text{ mg} \cdot \text{kg}^{-1}$  and median fruitiness of ‘Lecciana’ oils is  $4.4/7.0$  (Table 8). These are much higher values of those reported for Arbequina and Arbosana, the cultivars commonly planted in SHD olive orchard [47,55,56]. Moreover, ‘Lecciana’ oils fall into the category ‘nutraceutical EVOOs’ and so they can benefit from the specific functional health claim (Regulation EFSA No. 432/2012).

**Table 8.** Total polyphenols content ( $\text{mg}$  caffeic acid  $\cdot \text{kg}^{-1}$ ) and fruitiness median of ‘Lecciana’ oils during ripening (DAFB = days after full bloom). Mean values of two subsequent seasons ( $\pm$ SE). Lower case letters denote statistical differences at  $p = 0.05$  (Siegel-Tukey test).

Ripening Time (DAFB)		Polyphenols ( $\text{mg} \cdot \text{kg}^{-1}$ )	Fruitiness (n/7.0)
2015	2016		
177	180	$442.5 \pm 6.6$ c	$4.1 \pm 0.3$ b
191	194	$470.3 \pm 1.5$ a	$4.5 \pm 0.3$ a
206	209	$458.1 \pm 5.4$ b	$4.4 \pm 0.2$ a
220	223	$451.0 \pm 9.3$ b	$4.3 \pm 0.3$ ab

## 5. Conclusions

The challenges of olive intensification are linked to environmental, social and economical sustainability [57]. On the other hand, the cultivar is the key factor for sustainability of the olive SHD cropping system. ‘Lecciana’ is the first olive cultivar of Italian descent (cv.

Leccino) suitable for super high-density orchards, featuring all the vegetative and productive traits needed for efficient, sustainable olive growing intensification. Thanks to its low vigor, early bearing (3rd year after planting), high yield efficiency (about 0.5 kg of fruits per cm<sup>-2</sup> of trunk section area), good fruit size (3.5 g), ‘Lecciana’ could be planted with tree densities over 1,200 trees per hectare for efficient continuous mechanical harvesting. The new cv. Lecciana could be included in the Italian PDO regulations and it can yield *Made in Italy* oils with a reduction of 50% of production costs with respect to the best current intensive orchards, thanks to possible full mechanization of cultivation operations [58]; so it should help to support the olive oil supply chain economic sustainability. Moreover, the ‘Lecciana’ oils fall into the category ‘nutraceutical EVOOs’ which can benefit from the specific functional health claim (Regulation EFSA No. 432/2012); in addition, its fatty acids profile is very close to the reference values of the Italian monovarietal extra virgin olive oils.

Medium-term field observations in different growing areas (data not shown) indicate that ‘Lecciana’ is much more tolerant to frost and olive leaf spot than cv. Arbequina. On the contrary, olive fly infestation of ‘Lecciana’ olives seems to be slightly higher than those of cultivars Arbequina, Arbosana and Koroneiki, considering its bigger fruit size. Resistance/tolerance to *Xylella fastidiosa* subsp. *pauca* (OQDS disease) is currently under evaluation.

Finally, as SHD planting systems seem to reduce water footprint and other agronomical inputs [7–9], the diffusion of cv. Lecciana in new orchards could represent a very interesting approach to enhance the environmental sustainability in olive growing intensification as well.

## 6. Patents, Diffusion and Availability

A European Community plant variety patent (CPVO n. A201702935) for ‘Lecciana’ has been submitted in 2017 for and it enjoys a U.S. plant patent since 2019 (US PP no. 30,208 P2). Now it is submitted for registration in Australia, Turkey, Israel, Chile and Morocco. Up until 2020, about 2 million plants have been spread, 40% in Spain, 45% Italy, 15% Portugal and around 1,200 hectares have been planted. In other countries, plantations are still at the pre-commercial level. No licenses have been given at nurseries for the multiplication or propagation of the variety and only the Agromillora subsidiaries have it. Nursery plant production of more than 1 million plants per year is expected for the next few years.

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